REMARKS

Amendments to the Claims:

Claims 67-68, 77-78, 80-87, 89-94, 96, 102, 104-105, and 125-138 are pending in the application. Claims 87 and 87 were withdrawn from consideration. Claims 67-68, 77-78, 80-87, 89-94, 96, 102, 104-105, and 125-137 were rejected, and Claim 138 was objected to. By the foregoing amendment, Claims 67 and 136 have been cancelled, and Claims 68, 77, 78, 80, 84, 87, 90, 125-129, 137 and 138 have been amended. New claim 139 has been added. Support for new Claim 139 can be found at page 20, lines 25-26, for example.

Objection to Claim 138:

Claim 138 has been objected to as being dependent upon a rejected base claim, but the Office action indicated it would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims. Claim 138 has been amended to include the limitations of claim 90 prior to this amendment.

Rejection of Claims 67-68, 77, 80-84, 126-129 and 137 Under 35 U.S.C. § 102(e):

Claims 67-68, 77, 80-84, 126-129 and 137 are rejected under 35 U.S.C. § 102(e), on the basis that these claims are anticipated by Lauffer, et al., (US Publication No. 2001-0053539). The rejection asserts that Lauffer, et al., teach human erythropoietin (EPO) conjugated to various portions of an immunoglobulin (Ig), preferably the constant region, joined at the hinge region. The rejection contends that Lauffer, et al., teach an example which includes the penultimate aspartate residue of EPO in a BamH1 fusion. It is asserted that the fusions of Lauffer, et al., meet the limitation of "without an intervening linker", because the present claims do not define the sequences for the Ig domain and the EPO domain, and because the specification allegedly teaches that both EPO and Ig domains include "active variants" of the protein. The rejection apparently acknowledges that the fusions of Lauffer, et al., contain amino acids that are not normally present in natural human EPO or natural human IgG1, but contends that they can be considered variants of EPO and Ig. With respect to activity, the rejection asserts that this is an inherent feature of the fusion protein.

Applicant does not acquiesce in this rejection; however, in the interest of expediting prosecution, Claim 67 has been cancelled. Claims 68, 77, 80, 84 and 127-129, which were dependent on claim 67, have been amended to depend from Claim 125, which was not rejected under this section. Claims 81-83 remain dependent on amended Claim 80. Claims 126 and 137 have been amended to recite "wherein the fusion protein comprises the natural erythropoietin amino acid sequence and the natural immunoglobulin domain amino acid sequence at the junction of the fusion protein," which is a recitation appearing in Claim 125, but not Claim 67. In view of the foregoing amendments, withdrawal of the rejection of Claims 67-68, 77, 80-84, 126-129 and 137 under 35 U.S.C. § 102(e) is respectfully requested.

Rejection of Claims 90-94, 96, 104, and 130-136 Under 35 U.S.C. § 103:

Claims 90-94, 96, 104 and 130-136 are rejected under 35 U.S.C. § 103, on the basis that these claims are unpatentable over Lauffer, et al., in view of Mapelli, et al. (U.S. Patent No. 5,519,115). The rejection reasons that Lauffer, et al., teach an EPO-Ig fusion protein as discussed in the rejection under 35 U.S.C. § 102(e) above. It is acknowledged that Lauffer, et al., do not teach conjugating EPO and Ig with a linker between 2 and 7 amino acids in length, including 2, 4 and 7 amino acid linkers, where the linkers consist of glycine and/or serine residues. However, the rejection contends that Mapelli, et al. teach the use of small bridges of 5 amino acids or less in the construction of oligopeptides, and the use of glycine and serine in such linkers. The rejection characterizes Mapelli, et al. as teaching that linkers that hinder monomer interactions are generally greater than 5 amino acids in length. Therefore, it is asserted that it would have been obvious to combine the teachings of Lauffer, et al., and Mapelli, et al. because small bridges of 5 amino acids or less would be viewed as advantageous, and because there would be a reasonable expectation of success at making the fusions.

The rejection of Claims 90-94, 96, 104 and 130-136 under 35 U.S.C. § 103 is respectfully traversed.

The teachings of Lauffer, et al., are as follows. In Example 3 of US20010053539, Lauffer, et al., hypothesize construction of an EPO/IgG1-Fc fusion wherein the C-terminus of EPO is truncated and then joined to the N-terminus of an immunoglobulin domain with a Pro-

Glu linker sequence. It appears that the authors may have created the DNA constructs encoding this hypothetical fusion protein, but we note that they do not present any data demonstrating that an EPO/IgG-Fc fusion protein was expressed, purified or tested for biological activity *in vitro* or *in vivo*.

The Lauffer, et al., construct will always result in a fusion protein containing a peptide linker, with a minimum peptide linker sequence of Pro-Glu separating EPO and IgG-Fc, as explained in detail below. The vector used for expression of the EPO-IgG fusion proteins was designed to take advantage of the intron/exon structure of the IgG1 gene. In Lauffer, et al.'s expression vector, the Epo gene and the IgG-Fc gene are not contiguous; they are separated by an intron. Thus the transcribed mRNA must undergo an RNA splicing event to create an RNA capable of being translated into a contiguous EPO/IgG-Fc protein. The RNA splicing recognition sequences are very specific sequences of about 10 nucleotides, which limit the amino acid sequence possibilities at the junction of EPO and IgG-Fc. It is not possible to modify intron donor recognition sequences to encode different amino acids, and still retain the ability to function as a splice donor sequence.

The authors state that the EPO fusion gene was constructed using the same methods that were used to construct the IL-4 Receptor-Fc fusion protein described in their Example 2 (¶[0042]).

The expression vector described in Example 2 is plasmid pCD4Egamma 1 (¶[0032]), the sequence of which is given in Table 2 of European patent EP 0 325 262 A2 (¶[0019]). This expression vector contains the CD4 coding region separated by an intron from the human IgG1 constant region gene encoding the Hinge, CH2 and CH3 domains of human IgG1 (Example 1 of European patent EP 0 325 262 A2; see page 10, lines 56-57). As described in Example 1 of European patent EP 0 325 262 A2, page 10, lines 35-45, the authors state that they inserted a synthetic splice donor recognition sequence (GGATCCGAGGGTGAGTACTA) into the ESP I site upstream of the IgG Hinge encoding region of this plasmid, creating expression vector pCD4E gamma1. The splice donor sequence was modified to include a Bam HI recognition site (GGATCC) at its 5' end and a Hind III site (AGTACT) at its 3' end. The IgG-Fc Hinge region

exon begins about nucleotide 1666 of Table 2 (the amino acid sequence of the IgG1 hinge region begins EPKSCD, which is shown under the nucleotide sequence.)

More specifically, as stated on page 10 of Example 1 of EP 0 325 262 A2, the intron donor DNA sequence inserted at the Esp I site is GGATCCCGAGGGTGAGTACTA. The Bam HI (GGATCC) and Hind III (AGTACT) recognition sequences are underlined. The way the fusion gene is set up the GAT of the splice donor sequence is the GAT encoding Asp-165 of EPO. RNA splicing occurs between the GG nucleotide pair (indicated in bold type). Thus, any RNA produced by this method must include the nucleotide sequence GAT CCC GAG G', which encodes aspartic acid (encoded by GAT) followed by a two amino acid peptide linker comprising proline (encoded by CCC) and glutamic acid (encoded by GAG). This amino acid linker (DPE) is shown at the end the CD4 coding region in Table 2 of European patent EP 0 325 262 A2 (underneath nucleotides1298-1307). The final G at the splice junction is joined via mRNA splicing to the AG at the intron splice acceptor sequence (CTGCAGAGCCC), splicing occurs in between the GA nucleotides indicated in bold; the splice sequence is located at about nucleotide 1666 of Table 2) to create a GAG codon, which encodes the first glutamic acid (E) residue of the IgG1 hinge domain. Thus this method, if it does work, will always result in an EPO/IgG-Fc fusion protein containing at least a Pro-Glu amino acid peptide linker in between the EPO and IgG hinge region. In fact, in Table 2 one can see that the CD4 gene fusion encodes these amino acids (the last amino acids of the CD4 coding region located at about listed in Table 2 at about nucleotides 1298-1307).

In Example 3, to create the EPO-Fc fusion gene, the CD4 coding region was deleted and the EPO cDNA from the pCES plasmid was subcloned into the BamHI site of pCD4Egamma1. Lauffer, et al., describe oligonucleotides used to PCR amplify the EPO gene from plasmid pCES. Again, the oligos are designed to delete the C-terminal arginine residue of EPO (amino acid residue 166) and terminate the EPO coding region at the next to last amino acid of EPO, Aspartic acid-165. A Bam HI site is added following Aspartic acid-165 to facilitate joining to an exon donor recognition sequence in the expression plasmid. Bam HI has a recognition sequence of GGATCC and aspartic acid has a codon sequence of GAT. Thus, arginine-166 was deleted so that the GAT codon of Asp-165 could be incorporated into the Bam HI recognition sequence.

Again, it is also not possible to use the method of Lauffer, et al., to construct a fusion protein containing a peptide linker containing Serine and Glycine because of the sequence requirements of the RNA splicing sites.

Another way of saying this it that the EPO IgG-Fc fusion protein gene of Lauffer, et al., was constructed so that the EPO cDNA portion is flanked at its 3' end with an exon donor sequence, which is followed by an intron, which is followed by the exon encoding the IgG1-Fc Hinge region. Proper processing (deletion) of the intron sequences in the transcribed mRNA must occur for the EPO coding sequence to become contiguous with the IgG domain coding sequences, allowing translation of a contiguous EPO IgG-Fc protein. Thus, EPO and the IgG domain coding regions are not contiguous in Lauffer's constructs. We note that Lauffer, et al., do not demonstrate that correct mRNA processing occurs in their Epo IgG-Fc gene construct or that a functional EPO-IgG fusion protein is expressed.

In summary, Lauffer, et al., does not teach or suggest how to make a fusion protein with an intervening peptide linker containing residues other than Pro-Glu or which does not contain Pro-Glu.

Mapelli, et al. teaches the use of peptide linkers for the construction of a bridge to form *oligopeptides*, which are relatively small monomer peptides having antimicrobial activity that are linked together. Regarding the peptide linkers or bridges of Mapelli, et al., the references states that "the bridge in accordance with the present invention must be capable of promoting inter-monomer interactions amongst the monomers in a specific oligopeptide." (Col. 23, lines 16-19). An oligopeptide in accordance with the invention of Mapelli, et al., "is a functional protein containing at least two peptide subunits as the at least one first peptide monomer and the at least one second peptide monomer." (col. 5, lines 61-64). "Individual peptide monomers from which the oligopeptides are constructed are generally themselves antimicrobial peptides which are active against microbial pathogens." (col. 6, lines 13-16). The peptides are described as ranging from 4 to 37 amino acids (see Figure 1).

This reference is not at all directed to the production of fusion proteins in general, let alone an EPO-Ig fusion protein, which is a fusion of two large proteins with very different structures. EPO alone contains about 166 amino acids, which is much larger than the shorter

peptides used in the monomers of Mapelli, et al. The EPO-Ig fusion proteins are far more complex than the simple oligomers contemplated by the '115 patent, because EPO-Ig fusion proteins also interact to form disulfide-linked dimers, which can negatively affect their bioactivity. Thus, EPO and Ig proteins can not properly be considered to be peptide monomers as used by Mapelli, et al. Mapelli, et al., does not teach or suggest the use of full length proteins and requires that the bridge promote inter-monomer interactions. Mapelli, et al. teaches that this is desirable so that the oligopeptides can be transported across (plant) cell membranes (col. 23, lines 23-30) and provide the ability to protect against plant pathogens which is related to the ability to form aggregates (col. 23, lines 35-44). These concerns are not relevant in the production of EPO-Ig fusion proteins (which are not oligopeptides as defined by Mapelli, et al.) and thus there is no reason to look to Mapelli, et al., for guidance on a form of peptide linker in an EPO-Ig Fusion. Accordingly, Mapelli, et al. does not teach or suggest the presently claimed invention, either alone or in combination with Lauffer, et al.

Furthermore, Mapelli, et al., does not remedy the deficiencies of Lauffer, et al., and it is unclear what the result would be if Lauffer, et al., and Mapelli, et al., were combined. Lauffer, et al., requires a Pro-Glu linker in a fusion protein, while Mapelli, et al., emphasize the importance of a bridge providing or promoting inter-monomer interactions in an antimicrobial oligopeptide. There is no teaching or suggestion in Lauffer, et al., to use peptide monomers or a different linker and there is no teaching or suggestion in Mapelli, et al., to use larger proteins or to include the specific linker of Pro-Glu.

In contrast, Claim 90, as amended, is directed to a natural human erythropoietin (EPO) joined at its carboxy-terminus by a peptide linker to the amino terminus of an immunoglobulin domain that does not contain a variable region, wherein the peptide linker consists of between 2 and 7 amino acid residues, wherein the amino acid residues are selected from the group consisting of: glycine and serine (not Pro or Glu), and wherein said fusion protein has an EC_{50} within 4 fold of the EC_{50} of non-fused EPO, on a molar basis, in an EPO-dependent *in vitro* bioassay using a human UT7/epo cell line that proliferates in response to EPO. This combination of features is neither taught nor suggested by the combination of Lauffer, et al., and Mapelli, et al.

In view of the foregoing remarks, withdrawal of the rejection of Claims 90-94, 96, 104 and 130-136 under 35 U.S.C. § 103 is respectfully requested.

Rejection of Claims 133-135 Under 35 U.S.C. § 103

Claims 133-135 have been rejected under 35 U.S.C. § 103, on the basis that the claims are unpatentable over Lauffer, et al., in view of Mapelli, et al. and further in view of Qiu et al. The Examiner refers to the arguments regarding Lauffer, et al., and Mapelli, et al. discussed above, and acknowledges that neither Lauffer, et al., or Mapelli, et al. teach a peptide linker that consists of 7 amino acids and is composed of serine and/or glycine. The rejection asserts that Qiu et al. teach the use of a sequence comprising 3 to 7 glycine residues for the construction of dimeric EPO. Therefore, the rejection reasons that it would be obvious to combine the teachings of Lauffer, et al., Mapelli, et al. and Qiu et al. because Qiu et al. teach that the use of 3-7 amino acid linkers can confer functional confirmation to the EPO dimer, and that the combined teachings provide a reasonable expectation of success of linking the components without affecting the structure and biological activity of the fusion.

The rejection of Claims 133-135 under 35 U.S.C. § 103 is respectfully traversed. The teaching of Lauffer, et al., Mapelli, et al., are taught above. Briefly, Lauffer, et al., teaches that a Pro-Glu linker should be used with an EPO protein, and does not teach or suggest alterations in the linker. Mapelli, et al., teaches oligopeptides made of individual peptide monomers that are active against microbial pathogens and connected by bridges. The EPO-Ig fusion protein of Lauffer, et al., is not an oligopeptide as defined by Mapelli, et al., and thus there is no reason to look to Mapelli, et al., for guidance on a form of peptide linker in an EPO-Ig Fusion. Furthermore, Lauffer, et al., requires a Pro-Glu linker in a fusion protein, while Mapelli, et al., emphasize the importance of a bridge providing or promoting inter-monomer interactions in an antimicrobial oligopeptide. There is no teaching or suggestion in Lauffer, et al., to use peptide monomers or a different linker and there is no teaching or suggestion in Mapelli, et al., to use larger proteins or to include the specific linker of Pro-Glu.

Qiu, et al., does not remedy the deficiencies of Lauffer, et al. or Mapelli, et al. Qiu, et al., reported that EPO-EPO fusion proteins joined by peptide linkers of 3-7 glycine residues have

significantly reduced biological activities (4-10-fold) relative to wild type EPO. Thus, Qiu, et al., does not teach that linking the components will not affect the structure and biological activity of the fusion, as asserted by the Office action.

Furthermore, the literature teaches that the size and sequence of peptide linkers can dramatically affect bioactivities of fusion proteins. Moreover, much of the literature, including that previously cited by the Examiner, teaches that peptide linkers should be longer than the presently claimed linkers. For example, in the reference of Robinson et al., *Proc. Natl. Acad. Sci. USA* 95:5929-34 (1998), only linkers with 13 or more amino acids resulted in biologically active fusion proteins. Linkers with 3, 8 or 9 amino acids were inactive in the fusion of Robinson et al. and linkers with 11 amino acids were only partially active. Thus, the disclosure of Robinson et al. teaches away from using linkers of less than 11 or 13 amino acids for creating biologically active Epo/IgG fusion proteins. As another example, Chang (U.S. Patent No. 5,723,125) describes alpha interferon/IgG-Fc fusion proteins joined by a peptide linker. Chang found that an alpha interferon fusion protein containing a 16 amino acid linker (GGSGGSGGGGSGGGGS) had 5-10-fold greater specific activity in anti-viral assays than a related alpha interferon/IgG-Fc fusion protein containing a smaller, 6 amino acid linker (GGSGGS) (see column 5, lines 43-50). Therefore, this reference teaches that the length of the linker can dramatically impact the biological activity of the resulting fusion protein, and further teaches that longer peptide linkers are preferred. This information and additional supporting information was previously submitted in the form of a Declaration under 37 C.F.R. § 1.132 of Dr. George Cox, a coinventor of the present invention. The art, taken as a whole, therefore, indicates that the effect of the length of the linker on the activity of a fusion protein is unpredictable.

In contrast, the present specification demonstrates the ability to produce the recited fusion proteins with substantially similar biological activities to the wild-type protein, which is not predicted by the combination of references cited by the Examiner.

In view of the foregoing remarks, withdrawal of the rejection of Claims 133-135 under 35 U.S.C. § 103 is respectfully requested.

Rejection of Claims 78, 86, 102 and 105 Under 35 U.S.C. § 103:

Claims 78, 86, 102 and 105 are rejected under 35 U.S.C. § 103, on the basis that these claims are unpatentable over Lauffer, et al., in view of Sytkowski (U.S. Patent No. 5,580,853). The rejection cites Lauffer, et al. for the reasons discussed above and acknowledges that Lauffer, et al., do not teach a composition comprising a dimeric EPO-Ig or a method of purifying such fusion protein. The rejection cites Sytkowski as allegedly teaching multimeric EPO covalently linked by thioether bonds. The rejection reasons that it would have been obvious to combine the teachings of Lauffer, et al., and Sytkowski because Sytkowski teaches that multimeric EPO has increased bioactivity, and that the combination provides a reasonable expectation of success at making such bioactive proteins.

The rejection of Claims 78, 86, 102 and 105 under 35 U.S.C. § 103 is respectfully traversed.

Claims 78 and 86 were formerly dependent on Claim 67, but now depend from Claim 125. Claim 125 as amended claims a fusion protein comprising a human erythropoietin protein joined without an intervening peptide linker to a human immunoglobulin (Ig) domain that does not contain a variable region, wherein the fusion protein comprises the natural human erythropoietin amino acid sequence and the natural human immunoglobulin domain amino acid sequence at the junction of the fusion protein. As noted above, Lauffer, et al., does not teach such a fusion protein; rather Lauffer, et al., hypothesize construction of an EPO/IgG1-Fc fusion wherein the C-terminus of EPO is truncated and then joined to the N-terminus of an immunoglobulin domain with a Pro-Glu linker sequence. Lauffer, et al., neither teaches nor suggests a fusion as claimed in Claim 125. Sytkowski does not remedy the deficiencies of Lauffer, et al. Sytkowski teaches chemical modification of EPO proteins to introduce thiol moieties that react to create EPO dimers. Sytkowski neither teaches nor suggests using EPO derivatives such as EPO fusions. Furthermore, there is no expectation of success in applying the method of Sytkowski to EPO/IgG1-Fc fusions. Sytkowski notes that polypeptides suitable for modification by the methods taught are "preferably monomeric polypeptides, which do not contain any free sulfhydryl groups," (col. 3, lines 44-47), and that wild type EPO does not contain any free sulfhydryl groups (col. 4, lines 59-61). In contrast, the fusion protein of

Lauffer, et al. does contain free sulfhydryl groups in the immunoglobulin portion of the fusion, which would likely interfere with the desired cross-linking reaction.

Thus, the combination of Lauffer, et al., and Sytkowski does not teach or suggest the invention of claims 78 and 86, since neither Lauffer, et al., nor Sytkowski provide for a fusion protein comprising a human erythropoietin protein joined without an intervening peptide linker to a human immunoglobulin (Ig) domain that does not contain a variable region, wherein the fusion protein comprises the natural human erythropoietin amino acid sequence and the natural human immunoglobulin domain amino acid sequence at the junction of the fusion protein, as required by these claims.

Claims 86 and 102 are dependent from Claim 90. The combination of Lauffer, et al., and Sytkowski also does not render these claims obvious. As noted above, Lauffer, et al., hypothesize construction of an EPO/IgG1-Fc fusion wherein the C-terminus of EPO is joined to the N-terminus of an immunoglobulin domain with a Pro-Glu linker sequence, whereas Claim 90 requires a peptide linker consists of between 2 and 7 amino acid residues, wherein the amino acid residues are selected from the group consisting of glycine and serine. Lauffer, et al., does not teach or suggest a full-length EPO and a glycine- and serine-containing peptide linker in the fusion protein. Sytkowski, et al., teaches only EPO-EPO dimers neither teaches nor suggests the use of EPO fusion proteins or glycine- or serine-containing linkers in fusion proteins.

In view of the foregoing remarks, the withdrawal of the rejection of Claims 78, 86, 102 and 105 under 35 U.S.C. § 103 is respectfully requested.

Rejection of Claim 125 Under 35 U.S.C. § 112, Second Paragraph

Claim 125 is rejected under 35 U.S.C. § 112, second paragraph. The rejection reasons that the phrase "the natural erythropoietin amino acid sequence and the natural immunoglobulin domain amino acid sequence" is unclear because without a sequence or specifying the species of the amino acid sequence (human, mouse, etc.), the metes and bounds of the claim allegedly cannot be determined. The second paragraph of Section 112 requires that the claims set out and circumscribe a particular area that applicants regard as their invention with a *reasonable* degree of precision and particularity.

Claim 125 has been amended to recite that the erythropoietin and immunoglobulin domain amino acid sequences are human sequences.

In view of this amendment, withdrawal of the rejection of Claim 125 under 35 U.S.C. § 112, second paragraph is respectfully requested.

Closing Remarks

Applicant believes that the pending claims are in condition for allowance. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for any needed extension of time and an authorization to charge all fees therefore to deposit account No. 19-1970, if not otherwise specifically requested. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to be charged to deposit account No. 19-1970.

Respectfully submitted,

SHERIDAN ROSS P.C.

By: /Darla G. Yoerg/
Darla G. Yoerg
Registration No. 48,053
1560 Broadway, Suite 1200
Denver, Colorado 80202-5141
(303) 863-9700

Date: May 14, 2008